AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
BY THE BIBLIOGRAPHIC SERVICE, JULY 12

ACTIVITIES OF COLONIAL ANIMALS

I. CIRCULATION OF WATER IN RENILLA[1]

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ONE TEXT FIGURE AND ONE PLATE (FOUR FIGURES)

INTRODUCTION

Although much attention has been devoted in the last few decades to the movements, reactions, and other activities of single individual animals among the coelenterates, such, for instance, as the sea-anemones and the jelly-fishes, very little effort has been directed toward the study of colonial forms, like the corals and the hydroids. At present it is quite impossible to state with any degree of certainty how, from the standpoint of functional interdependence, the individual zoöids in such a colony are related to the colony as a whole. For the solution of a question of this kind a well-circumscribed aggregate of large zoöids is needed. Such a condition is to be met with in the sea-pen, Renilla. The zoöids in this genus are relatively large, and the whole colony, though anchored in the sand, can be removed without damage and readily subjected to experimental study.

This genus, though restricted to the warmer seas, is distributed very widely within such limits. One of its largest species is Renilla amethystina Verrill, a form very abundant in the shallow waters about San Diego, California. This species was chosen for experimental investigation, and all the material used in this work was collected near the outlet of False Bay in the vicinity of La Jolla, California. I am under obligations to Dr. W. E. Ritter, Director of the Scripps Institution for Biological Research at La Jolla, for the opportunity of carrying out this work, and I am indebted to the staff of that institution, especially to the collector,

[1] Contributions from the Zoölogical Laboratory of the Museum of Comparative Zoölogy at Harvard College, no. 323.

Mr. P. S. Barnhart, for much help given me while I was working there.

Renilla amethystina was described originally by Verrill ('64 a, p. 29), and the group to which it belongs was subsequently monographed by Kölliker ('72). A detailed account of the structure and habits of Renilla amethystina was published by Eisen in 1876, and the development of the closely allied species, Renilla reniformis, was investigated with great fullness by Wilson ('83). Musgrave's experimental studies on living sea-pens appeared in 1909. Ten years later I published a brief account of the organization of Renilla amethystina based upon the study of the few specimens of this species available at La Jolla in 1916. In a measure this communication is a preliminary to the present investigations, which were carried out on much more abundant material in 1919 and which I shall present in two papers, the first dealing with the circulation of water in the colony and the means of expansion and contraction.

GENERAL HABITS OF RENILLA

The majority of sea-pens are elongated colonial organisms, one portion of whose axis, the stalk or peduncle, anchors the colony in the mud or sand of the sea bottom, while the other portion, the rachis, is held above the sea bed and carries the polyps. Renilla is peculiar in that its peduncle is a relatively soft, fleshy structure without an axial skeleton, and its rachis is spread out laterally at right angles to the axis of its peduncle. When in its natural position the peduncle of Renilla is sunk vertically in the sand on the surface of which the rachis rests horizontally. The rachis is a kidney-shaped or heart-shaped structure whose indentation marks the region at which the peduncle is attached. The lower surface of the rachis, that next the sand, is devoid of polyps, all of which are limited to the upper surface. In consequence of comparison with other sea-pens, some confusion exists in the various accounts as to the proper designations to be used for the two surfaces of the rachis of Renilla. In the older terminology for the sea-pens, that surface which carried the zoöids was called ventral, the opposite one dorsal. Accordingly, in Renilla the upper surface as the colony rests on the sand would be ventral, the lower dorsal, a perversion that recent writers have been disinclined to follow. To avoid confusion in this respect, I shall, therefore, not use the terms dorsal and ventral, but I shall call the surface of the Renilla rachis that is uppermost in the resting position and that carries the zoöids superior and the opposite one inferior.

Renilla amethystina is commonly found in sand banks between high and low tide. When such a bank that has been covered for some time with a foot or so of water is closely examined, many of the heart-shaped colonies of this species will be discovered fully expanded and spread out upon the surface of the sand (fig. 1). In Renilla amethystina the maximum diameter of the colony may reach 8.5 cm. and the largest zoöids may rise from the surface of the rachis to the height of 5 or 6 mm. Notwithstanding the fact that Renilla is so bright a purple as to justify the popular name of sea-pansy, the colony in its resting position is so commonly covered with a thin layer of sand as to make it easily overlooked. This peculiarity is enhanced by the fact that the zoöids, which are almost always well above the level of the sand, are transparent or at most slightly grayish in tint.

As the tide recedes Renilla contracts and withdraws gradually into the sand so that with the disappearance of the last of the water the animal leaves a mark on the sand not unlike that of a miniature horseshoe (fig. 2). These marks serve as sure indications to the collector of the presence of Renilla. They are quickly obliterated by the returning tide, whereupon Renilla reexpands to assume the form already described. These tidal responses were observed as early as 1864 by Fritz Müller.

The expansion and contraction of Renilla is accomplished, as might be expected, by taking in and discharging sea-water. Louis Agassiz ('50, p. 208) long ago observed that an expanded Renilla reniformis might thus temporarily have a diameter double that of its contracted state, and the same seems to be true of Renilla amethystina. A specimen of this species (fig. 3), whose rachis when fully expanded had a diameter of 6.5 cm. was made to contract completely (fig. 4), after which its diameter was found to measure 3.2 cm. Before contraction its volume was 27 cc. and after contraction this had been reduced to 3.2 cc. through the discharge of 23.8 cc. of water—a reduction of over 88 per cent of its volume. This very considerable change of form and volume was early observed by Müller ('64, p. 353), who pointed out its importance in specific descriptions in this genus. The complete contraction of a fully expanded Renilla may be accomplished under special stimulation in a minute or so. Its expansion, which necessitates that it shall refill itself with water, requires at least half an hour.

Under natural conditions probably the majority of Renillas expand and contract as already indicated with the flowing and ebbing of the tide. Specimens kept in sand-filled aquaria in the laboratory, however, even though continually under water, will at times contract, bury themselves in the sand, and remain thus hidden for considerable periods. In one instance a Renilla retreated under the sand and remained quiescently there for three and a half days, whereupon it was dug out and placed in sea-water. It then inflated itself and acted in other respects entirely normally. It is therefore possible for Renilla to remain contracted and quiescent for considerable periods, though this is probably not often the case under natural conditions.

INCURRENT AND EXCURRENT APERTURES

By what apertures water enters and leaves the body of Renilla is by no means certain. Even the number of kinds of openings in the body of this animal is still in dispute. At most four sets of such apertures have been distinguished. There are, first, the autozoöids, or ordinary polyps, generally scattered over the superior surface of the rachis. Each of these zoöids is provided with a mouth, which Agassiz ('50, p. 209) regarded as the chief means of entrance and exit of water for the colony as a whole. Next there are found among the autozoöids and imbedded in small masses of whitish materials groups of pores each one of which represents the mouth of a lateral siphonozoöid. These siphonozoöids were originally described by Verrill ('64 b, p. 12) as rudimentary individuals. According to Wilson ('83, p. 725), they are the chief inlets for Renilla, but Musgrave ('09, p. 472) regards them both as inhalent and exhalent apertures. The third kind of opening is a well-defined pore, the axial siphonozoöid. This lies on the superior surface of the rachis near its center and at the end of a smooth tract of integument that starts from the root of the peduncle. This pore was first recorded by Müller ('64, p. 354), who regarded it as the chief inlet and outlet for water in Renilla. Wilson ('83, p. 725), on the other hand, designated it as the exhalent zoöid for the colony. It probably corresponds to the group of four pores in the axis of the dorsal face of the rachis of Pennatula mentioned by Musgrave ('09, p. 454). Finally, there remains the problematic terminal pore supposed to be present at the distal end of the peduncle and regarded by various investigators as an inlet or an outlet aperture. These four classes of openings may now be considered in the order named.

The autozoöids

The true polyps or autozoöids of Renilla, as already stated, are disposed upon a somewhat irregular radial plan over the superior surface of the rachis of this animal. Near the center of the rachis they are relatively large and, when fully expanded, they may measure as much as 6 mm. in height. Toward the outer edge of the rachis they become smaller and more numerous.

Each autozoöid carries at its distal end eight pinnate tentacles surrounding an elongated slit-like mouth. One autozoöid lies in the axis of the rachis and opposite the peduncle from the axial siphonozoöid. This autozoöid, as has been shown by Wilson ('83) represents the original polyp produced from the egg, from which the remaining polyps of the colony have been formed by budding. The axis of the mouth of this autozoöid agrees in direction with that of the colony as a whole. Those of the mouths of the other autozoöids are in a similar manner in line with appropriate lateral axes. Marshall ('83, p. 140) showed that in the lateral autozoöids of the sea-pens the angle of the mouth that was turned away from the chief colonial axis, the abaxial angle, leads into a ciliated groove, the sulcus or siphonoglyph, whose cilia lash inward and might therefore be expected to carry water into the colony. In a corresponding fashion the axial angle of the mouth leads to a pair of mesenteric filaments whose cilia beat outward. Thus the autozoöids are so organized that they might well serve as a means for the entrance and exit of water for the colony as a whole, as originally maintained by Agassiz ('50, p. 209) for Renilla and as has recently been claimed by Musgrave ('09, p. 472) for sea-pens in general.

To what extent the autozoöids of Renilla can serve the colony for the entrance and escape of water may be inferred from the following observations and tests. Wilson ('83, p. 728) noticed that in Renilla reniformis the eggs and sperm were discharged through the mouths of the autozoöids. The same is true of Renilla amethystina. In the early part of August, 1919, many females of this species were found to be spawning. From time to time an egg was seen to rise through the oesophagus of an autozoöid and to shoot from its mouth into the surrounding sea-water. The time required for the passage of the egg through the length of the zoöid and for its discharge to the exterior was about fifteen seconds. As the egg first came into sight at the base of the zoöid it was spherical in form and its upward motion was relatively slow. After it had passed the middle of the zoöid, its rate quickened and it became elongated in form, its long axis corresponding to that of the zoöids. It emerged from the mouth as though forced out under pressure, suggesting a sphincter-like action for the distal half of the zoöid. The whole operation gave the impression of a process under the control of cilia which were less effective in the proximal than in the distal half of the zoöid, where the egg appeared to be under some pressure very likely of a muscular origin. The cilia most probably concerned in this operation are those of the mesenteric filaments, which, as already stated, are believed to beat toward the exterior.

In a similar way feces were seen from time to time to be discharged from the mouths of the autozoöids. If a fairly thick mixture of carmine and sea-water or of india ink and sea-water is injected into the central spaces of the colony, in about an hour thereafter long, thin, vermiculate lines of red or black material, in accordance with the injection used, were slowly discharged from the mouths of many of the autozoöids.

If a strong solution of methylen blue in sea-water is injected into an expanded Renilla, in a very few minutes the colored fluid may be seen in one or more of the eight mesenteric chambers of the autozoöids and even in the tentacles connected with these chambers, and in a very short time the solution may be discharged as a blue cloud from the mouths of these zoöids. These several lines of observation make it perfectly clear that the autozoöids of Renilla may discharge from the colony to the exterior both solids and fluids.

In a similar way inward movements may be demonstrated in these zoöids. If a very small piece of crab meat is dropped on the mouth of an expanded autozoöid, the tentacles quickly close over it and it is passed into the oesophagus. The distal half of the zoöid then contracts slowly but vigorously like a sphincter, and the bit of meat passes very gradually through the proximal half of the zoöid till, in about five minutes after it was placed on the lips, it disappears in the depths of the animal. After this disappearance the zoöid gradually elongates and assumes its normal proportions. The operation in this instance, like that in the discharge of eggs, feces, etc., appears to depend upon ciliary action, but whether in Renilla, as in the sea-anemones, the movement is due to a reversal of the usual outward stroke of the cilia in the region of the lips or whether the food is swept in by the normal action of the cilia of the sulcus could not be ascertained. In any event the autozoöid of Renilla can carry solid material inward as well as outward through its oesophagus.

If the distal end of an autozoöid of Renilla is flooded with carmine or methylen blue in sea-water, it should be easy to see whether inhalent or exhalent currents are normally present. As a matter of fact, all tests of this kind failed to demonstrate under ordinary circumstances any currents either outward or inward at the mouth of the autozoöid. If under such circumstances the distal end of a zoöid is quickly cut off by a single stroke of a pair of fine scissors, a momentary outward gush of water takes place, during which the beheaded polyp contracts. Presently it reexpands with its distal wounded end fully distended and closed as by a puckering string,—further evidence of the sphincter-like action of the distal half of the zoöid. All these operations occur in such a way that it is clear that the fluid inside the colony is ordinarily under a slight continuous pressure. Nevertheless, both methylen blue and carmine gave under ordinary conditions not the least evidence of water currents emerging from the mouths of the autozoöids or entering them, and I am forced to conclude that while the autozoöids of Renilla are effective means of introducing solid materials into the colony and of discharging like substance from it, they play no important part in the exchange of sea-water whereby the colony as a whole expands or contracts. Under considerable and unusual pressure they may aid somewhat in the discharge of water, but even in this respect they are certainly insignificant in their action as compared with other structures to be considered presently. So far as sea-water is concerned, they are, in my opinion, neither the chief inlets or outlets of the colony (Agassiz, '50, p. 209) nor even subordinate ones (Musgrave, '09, p. 472).

The lateral siphonozoöids

Scattered irregularly among the autozoöids of Renilla are groups of small whitish bodies, the lateral siphonozoöids, first clearly recognized by Verrill ('64 b, p. 12). Each group consists of a variable number of pores, the lips of which exhibit, as a rule, eight lobes, thus indicating that each pore represents a single zoöid. Not infrequently pores are met with whose size and structure suggest that they may be aborted autozoöids, but even in the extremes of these cases the whole body has a greater resemblance to a siphonozoöid than to an autozoöid, and since actual transitional forms between the two kinds have never been found, it is probable that in Renilla the two classes, the autozoöids and the siphonozoöids, are entirely distinct.

Each siphonozoöid pore is surrounded by a ring of whitish substance, in color and texture like that at the base of the autozoöids, and the whole group of siphonozoöids is set in a field of yellowish crystalline material. If finely divided carmine in sea-water is flooded over the surface of an expanded Renilla, sooner or later much of it will be found to have collected at the pores of siphonozoöids. And if such a preparation is closely watched under a hand lens, very small pieces of carmine will often be seen darting into the siphonozoöid pores. If a piece of the superior surface of the rachis is separated from the rest of the colony by a cut approximately parallel to that surface, and if the spaces connected with the siphonozoöids and exposed on its under face are closely watched, small carmine particles are seen to shoot out from these spaces and into the surrounding sea-water. As such preparations show, the siphonozoöids ordinarily conduct water from the exterior to the interior and such currents are generated within the siphonozoöid itself. The currents are without doubt the results of ciliary action.

Not infrequently siphonozoöids will be found in which no currents can be demonstrated. If these are watched for a time, currents will sooner or later be seen in them, the whole condition recalling strikingly that of the lateral pores of sponges whose currents, though produced by the flagellated cells, are controlled by the sphincters or other like devices which close and open these pores. In my opinion, the siphonozoöid currents in Renilla are controlled by some such device.

If methylen blue in sea-water is injected under pressure into the internal spaces of a living Renilla, small amounts of this fluid will be seen oozing from a few of the siphonozoöids. If the peduncle of a Renilla is cut open and a vertical glass tube tied securely into the cut, the end of the tube and the animal being under sea-water, it will be found that a pressure of some 5 or 6 cm. of water is necessary to drive a methylen-blue solution through the siphonozoöid pores to the exterior. This pressure may be taken, therefore, as the pressure necessary to counteract that by which the siphonozoöid currents are produced. The escape of methylen-blue solution thus brought about at the siphonozoöids is never very general and never large in amount. It indicates that the siphonozoöid pores are not provided with any effective valve-like parts whereby the currents are limited in direction and that, while the currents naturally set in through the siphonozoöids with great freedom, they take the opposite course, only under considerable stress as though they were working against the natural mechanism of the pore. A close and often repeated inspection of the siphonozoöids of Renilla when in a normal resting state failed to elicit the least evidence of natural currents running through these apertures in an outward direction. No carmine particles were ever seen wafted outwardly from these zoöids and methylen-blue solutions were never observed to be swept away from them. Excepting under an internal pressure of 5 or more cm. of water, I have never seen anything emerge from the siphonozoöid pores. This condition is probably associated with the fact, long ago pointed out by Wilson ('84, p. 18), that in Renilla the siphonozoöids are devoid of dorsal mesenteric filaments and hence produce no outward currents, their single ciliated organ being a siphonoglyph, by which water is carried inward. In many pennatulids the siphonozoöids possess dorsal mesenteric filaments (Lightbown, '18) as well as siphonoglyphs, and may therefore exhibit exhalent as well as inhalent activities. This condition probably explains the difference between my observations and those of Musgrave ('09, p. 455), who found in the siphonozoöids of Pennatula good evidence for excurrent as well as incurrent action, whereas in Renilla I found no trace of excurrents whatever. My observations completely confirm Wilson's statement ('83, p. 725) that the siphonozoöids of Renilla are the chief inhalent apertures for the colony.

The axial siphonozoöid

This large zoöid in the chief axis of the Renilla colony and near the center of its rachis was shown Müller ('64, p. 345) by his little daughter, who saw a jet of water spurt out of it when she picked up a living Renilla from the sea. This observation can be confirmed by anyone to whom living material is available. On taking up an inflated specimen, contraction at once occurs and water is discharged in the form of a fine jet from the axial siphonozoöid. The same form of discharge can be observed in animals under water. If an inflated Renilla in a basin of sea-water is touched slightly or the whole basin jarred, contraction begins, the membranous edges of the axial siphonozoöid withdraw, leaving the aperture freely open, through which emerges a jet of water strong enough to ruffle the surface of that in the basin. If a number of expanded Renillas are watched in a shallow aquarium, one or other of them will be seen to contract from time to time—an operation that is invariably accompanied by the opening of the pore of the axial siphonozoöid and the free discharge of water through it.

The internal pressure necessary to bring about the opening of this pore may be tested in the following way: if a vertical glass tube be tied securely into the superior canal of the peduncle of a submerged Renilla—a canal that leads directly to the inner end of the axial siphonozoöid—and the tube be filled with a solution of methylen blue in sea-water, it can be shown that the axial pore will not open till the internal pressure has reached 20 to 25 cm. of water. Since this canal communicates not only with the axial pore, but also with the pores of the lateral siphonozoöids, this experiment is always accompanied with the oozing of the methylen-blue solution at these pores, but even under the high pressure used this is never profuse, and it is very probable that the recorded pressures of from 20 to 25 cm. of water represent the real pressures necessary to open the axial pore. It is thus clear that a much higher pressure is needed to open this pore than to reverse the currents of the lateral siphonozoöids, but it is also clear, since the volume of the colony does not begin to show an appreciable diminution till the axial pore is opened, that the lateral siphonozoöids are of no great significance in discharging water from the colony as a whole. This conclusion is supported by the observation that when a Renilla begins to contract, methylen-blue solutions or carmine discharged over the superior face of its rachis give no evidence of outward currents from the pores of the lateral siphonozoöids or from the mouths of the autozoöid. If, under such circumstances, water does escape through these apertures, it must be very small in amount.

Repeated tests with carmine and with methylen blue of the axial siphonozooid in resting Renillas failed invariably to give any evidence of inward currents, and the same was true of individuals that were in process of inflation. I, therefore, cannot agree with Müller ('64, p. 354) in regarding this aperture as an inlet, though I fully concur in his opinion that it is an outlet, and from the abundance and freedom of its discharge I believe Wilson ('83, p. 725) to be correct in regarding it as the chief exhalent aperture of Renilla.

The terminal pore of the peduncle

The question of the presence of a terminal pore at the distal end of the peduncle of the sea-pen has been a matter of much dispute. The historical aspect of this subject has been well summarized by Musgrave ('09, p. 443). To some of the older naturalists, who regarded the sea-pens as single animals, the terminal pore of the peduncle appeared to be the mouth of the animal. To others its very existence was doubted. Müller ('64, p. 357) claimed that there was a pore at the tip of the peduncle of Renilla, and a similar condition was reported by Schultze ('64, p. 360) for Pennatula. Kölliker ('72, p. 86) was unable to confirm these findings on alcoholic material, but in fresh specimens of Renilla amethystina Eisen ('76, p. 13) demonstrated a terminal pore by pressing the fluid contents of the peduncle till they spurted from the tip of that structure. Musgrave ('09, p. 452) has given very conclusive evidence for the presence of a group of pores about the distal end of the peduncle of Pennatula. These pores, she believes ('09, p. 457), may serve both as inhalent and exhalent apertures. Thus the later evidence very generally favors the view that the sea-pens, including Renilla, possess terminal pores on the peduncle.

My own experience agrees very fully with that of Eisen. Although I have picked up many hundreds of inflated Renillas and have many times seen the jet of water issue from the axial siphonozoöid, I have never once seen under such circumstances water spurt from the tip of the peduncle—a condition that led me in the beginning to doubt the existence of a terminal pore.

Only when the peduncle is pinched between the fingers does a fine jet of water issue from its tip. Hence it is necessary to consider whether this aperture is due to artificial rupture or is a natural one and, if it is such, whether it is an exhalent or inhalent aperture.

To determine whether or not a natural pore exists at the tip of the peduncle of Renilla, the following tests were made. In ten fresh, vigorous animals the peduncle was pinched between the fingers till a jet of water appeared and the exact point of issuance was then noted. In six instances the jet issued with great regularity at the very tip of the peduncle. In two of the ten specimens two jets appeared one at the tip and the other on the side of the peduncle, but near the tip. In the remaining two the jets were from the side. In all instances the lateral jets were less regular than the terminal ones, as though the lateral jets issued through small ruptures and the terminal ones through a natural pore.

Peduncles from ten fresh animals were then cut off and put in sea-water to which was added some magnesium sulphate. After three hours these peduncles were attached at their cut ends one by one to the tip of a vertical glass tube which was made to dip under the surface of a bowl full of sea-water. Sea-water colored with methylen blue was then run into the tube till it issued through the peduncle. In six instances it emerged through the exact tip of the peduncle in a very thin well-defined stream and when the column of colored water in the tube showed a height of from 12 to 15 cm. In the four remaining peduncles it emerged as an irregular stream in each case from the side of the given peduncle.

Normal peduncles, unanesthetized with magnesium sulphate, were then tried in the same way. In the most resistant of these the colored water flowed from the tip of the peduncle when the fluid in the tube reached the height of 115 cm. and continued to flow till it fell to 40 cm., when the opening closed. In the least resistant the flow began at 80 cm. of water pressure and was checked at 20 cm. These observations, like those based on the position of emergence of the jets, favor the view that in Renilla there is a natural terminal pore on the peduncle.

Although it may be possible that there are individual differences in Renilla and that some specimens possess terminal peduncle pores and others do not, it seems to me more probable that such pores are characteristically present and that they open normally only under considerable internal pressure such as is represented by a column of sea-water approximately a meter in height—a pressure that may at times rupture the wall of the peduncle laterally rather than force open the pore.

Aside from the discharge of sea-water under unusual pressure, I have never seen any evidence that the terminal pore of the peduncle is an exhalent aperture. In all experiments with Renilla in which carmine or methylen blue have been used I have never observed anything that led me to suppose that water was being discharged from this pore. When a fully inflated peduncle on a living Renilla is quickly and completely ligated about midway its length and it is thus left distended and still attached to the colony, it will retain its inflated condition unchanged for hours, though when a small hole is made in it by the prick of a pin it collapses at once. I therefore believe it improbable that the terminal pore of the peduncle serves as an exit for water except under very unusual pressure.

The opinion that it is an inhalent aperture is still more problematical. I have repeated on Renilla Musgrave's experiment ('09, p. 456) on Pteroides in which the peduncle of this animal was immersed in a colored fluid. A deeply colored solution of methylen blue in sea-water was prepared and four narrow-necked flasks were filled with it. Into the neck of each flask the peduncle of a Renilla was inserted the rachis of the animal resting on the edges of the mouth of the flask. Each flask with its contained fluid and Renilla was then cautiously sunk in a vessel of sea-water so as to cause as little mingling of the methylen-blue solution and the sea-water as possible. After the preparations had been standing ten hours the Renillas were removed, rinsed gently, and their peduncles examined. The outer surfaces of these parts were stained deep blue, but, contrary to the results obtained by Musgrave on Pteroides, no blue fluid or blue staining could be found inside the peduncles, showing that the terminal pore had not served as an inlet.

Six specimens of Renilla were allowed to discharge their contained water, and their peduncles were firmly ligated at about midlength. Thus the distal half of the peduncle, though still connected with the rest of the colony by its wall, had its communication with the colony by its canals cut off. These specimens were then returned to sea-water to determine whether the distal portions of the peduncles could refill themselves. After twelve hours they were as unfilled as at the beginning of the experiment. After twenty-four hours two of the six had filled, but on cutting these from the colony in the region just proximal to that at which they were tied, they quickly emptied themselves by discharging their fluid contents at the cut end, showing that in these two instances the ligatures had failed to hold. In the four empty peduncles no such looseness was observable. This experiment was repeated on another set of ten animals and with similar results. For some time none of the peduncles filled, but after thirty hours three of them were found distended, and in all of these the ligatures had loosened. Apparently the peduncle, which is a highly muscular tube, is extremely difficult to ligate under water and by its continued activity eventually slightly loosens ligatures that under ordinary circumstances would have held.

The results of the experiments in ligating peduncles and in sinking them into colored fluid, in both of which the peduncles failed to fill themselves from the outside, lead to the conclusion that the terminal pore of that structure is not to be regarded as an inlet for sea-water. This conclusion accords with the fact that a Renilla immediately after its peduncle has been firmly ligated will fill itself with sea-water long before the ligature could have worked loose even to a small degree. Although I am convinced that the peduncle of Renilla possesses a terminal pore, I do not believe that it possesses inhalent and exhalent functions such as have been found by Musgrave ('09, p. 471) for the corresponding openings in other sea-pens. In Renilla, so far as I can judge, the terminal pore is at best an outlet opening serviceable only under excessive pressure.

Summary

From what has been stated in the preceding sections it appears that in a normally quiescent Renilla sea-water is entering the colony almost exclusively through the pores of the lateral siphonozoöids. A small amount may enter through the mouths of the autozoöids, but this at most must be very insignificant. There is no reason to suppose that water enters the colony through any other openings. In a quiescent colony water from time to time passes out through the pore of the axial siphonozoöid, and this is apparently the only method of exit under ordinary circumstances. Possibly an insignificant amount of water may pass out by the mouths of the autozoöids. If for any reason the fluid pressure within the colony becomes high, the currents of the lateral siphonozoöids may reverse and a small amount of water may escape through the pores of these zoöids. If the pressure increases still more, the terminal peduncle pore may eventually open and discharge. Such, however, would probably occur only under extreme pressure. In ordinary conditions the whole inflow of water is through the lateral siphonozoöids and the whole outflow through the axial siphonozoöid—a view long ago clearly set forth by Wilson ('83).

THE COURSE OF THE WATER WITHIN THE BODY OF RENILLA

The course of the water within the Renilla colony is dependent upon the system of canals within this animal. Agassiz ('50, p. 208) noted that both the peduncle and rachis of Renilla were more or less hollow and that their cavities communicated, and Verrill ('64, b, p. 12) was the first to recognize that the peduncle contained two canals each of which opened into extensive spaces in the rachis. Kölliker ('72, p. 85) showed that of these two canals one was superior in position and the other inferior and that the two communicated with each other near the distal end of the peduncle. Eisen ('76) confirmed these various statements and demonstrated further that the superior canal led directly to the pore of the axial siphonozoöid. He also claimed ('76, p. 12) that in the region of the rachis the peduncle of Renilla possessed four canals, two lateral ones in addition to the two characteristic of the peduncle. Wilson ('83, p. 726) corroborated all of Eisen's statements except that concerning the presence of the four canals, and showed that, although the superior canal of the peduncle communicated directly with the pore of the axial siphonozoöid, it was also connected with the cavities of the polyps as was the inferior canal. Much of this freedom of internal communication was demonstrated through injection by Musgrave ('09, p. 448) in the several sea-pens investigated by her.

In Renilla amethystina I studied the system of internal canals chiefly by means of injections. Two types of injecting fluids were used: first, solutions such as methylen blue in sea-water and, second, finely divided solids such as india ink in sea-water. The first kind would naturally pass through very minute apertures, the second only through those of a larger size. The injecting was accomplished under the pressure of a column of water, 10 to 15 cm. of which was usually found to be sufficient to drive the fluid forward.

If the peduncle of a Renilla is cut off close to its attachment to the rachis and the cannula of the injection tube tied firmly into the superior canal, the inferior canal being slit open throughout most of its length, it requires only a few centimeters of pressure to drive either a methylen-blue solution or an india-ink suspension down the length of the superior canal and through the numerous apertures of the septum between the two canals into the open inferior canal.

If, now, an additional ligature is applied to such a preparation at a point about one-quarter of the length of the peduncle from its tip, thus excluding this distal quarter from participating in the flow of the injection fluid, the fluid not only fills the remainder of the superior canal, but also flows over into the inferior one. On applying a third ligature to the peduncle about midway its length, it was found that, though the superior canal filled, neither the methylen-blue solution nor the india-ink suspension passed into the inferior canal. Hence it must be concluded that the pores in the peduncular septum by which the superior canal communicates with the inferior must be limited to about the distal half of this structure. When the cannula of the injection tube was tied into the superior canal of the distal quarter of the peduncle, the same freedom of communication between the two canals was demonstrated as when it was tied into the proximal end of the peduncle. It is, therefore, evident that the pores of the septum occur not only in the distal half of this structure, but also in its distal third and fourth quarters. They probably form a more or less continuous series in this region.

If the converse of the preceding experiments is tried, in that the injections are made into the inferior canal and the superior canal is cut open, fluid flows freely from the inferior into the superior canal, and it is fair to conclude that the pores in the septum between these canals are unprovided with valves that limit the direction in which fluid may pass. In the peduncle, then, the septum between the superior and inferior canal is impervious to injection fluids in the proximal half of its extent and freely pervious in its distal half. In this half the pores must be relatively large, for such coarse material as india ink is freely transmitted.

As Agassiz ('60, p. 208) long ago pointed out, the canals of the peduncle communicate freely with the spaces in the rachis. In all my work on the canals in the rachis of Renilla I have failed to find more than extensions and ramifications of the two canals of the peduncle of that sea-pen, and I am, therefore, unable to confirm Eisen's declaration ('76, p. 12) that where the peduncle joins the rachis in Renilla there are four canals, a number agreeing with that reported in the peduncles of many sea-pens. As I have worked upon the same species of Renilla that Eisen did, I suspect that in this particular his account is inaccurate.

If the peduncle of a Renilla is cut across about midway on its length and the injection cannula tied into the superior canal of the part which is still connected with the rachis, an india-ink injection can be made to flow through the superior canal and out at the pore of the axial siphonozoöid. The injection, as a rule, will not spread further. If methylen blue is used, a much more general injection is obtained. The methylen-blue solution flows first into the intermesenteric chambers of certain autozoöids, then oozes through some of the siphonozoöid pores, then discharges from the pores of the axial siphonozoöid, as the india ink did, and finally escapes from the mouths of some of the autozoöids. The conclusion to be drawn from these results is that the superior canal connects freely and directly with the opening of the axual siphonozoöid, as was first shown by Eisen ('76) and was subsequently confirmed by Wilson ('83), and that, contrary to my former opinion ('19, p. 503), it likewise connects, though very much less freely, with the autozoöids and siphonozoöids as maintained also by Wilson ('83).

When a corresponding form of injection is carried out on the inferior canal of the peduncle, a solution of methylen blue yields exactly the same results as when it is injected into the superior canal. India ink, however, remains limited to the inferior canal and only gradually makes its way out the mouths of some of the autozoöids. It fails entirely to reach the siphonozoöids or the axial pore. It is thus shown that the inferior canal in the rachis has freer communications with the autozoöids than with the lateral or axial siphonozoöids.

That the superior and inferior canal systems communicate in the rachis is not only proved by the results given in the preceding paragraphs, but may be demonstrated more directly in the following way. If an injection cannula is tied into the inferior canal of the peduncle in such a direction as to lead into the rachis and the superior canal is cut open from the peduncle to the axial pore, a methylen-blue solution on being injected into the inferior canal almost immediately appears in the open superior canal. If this procedure is carried out with india ink, instead of methylen blue, the ink fails to appear in the superior canal. The converse experiment of injecting into the superior canal and opening the inferior one yielded corresponding results except that there was often much loss of injection fluid at the axial pore. Both these lines of experimentation show that the superior and inferior canals in the body of the rachis are in communication with each other, but by openings not so large as those by which they communicate in the peduncle.

Sufficient facts have now been brought together to allow the formation of a reasonable hypothesis as to the course of the water through the Renilla colony. (In a resting expanded Renilla water is entering the animal through the innumerable pores of its lateral siphonozoöids (fig. A).) This water enters in consequence of the ciliary action of these zoöids. It fills the open spaces of the rachis and from time to time escapes to the exterior by passing either directly through the minute pores of the rachis tissue into the superior canal and out of the axial pore or indirectly by passing down the inferior canal of the peduncle to its

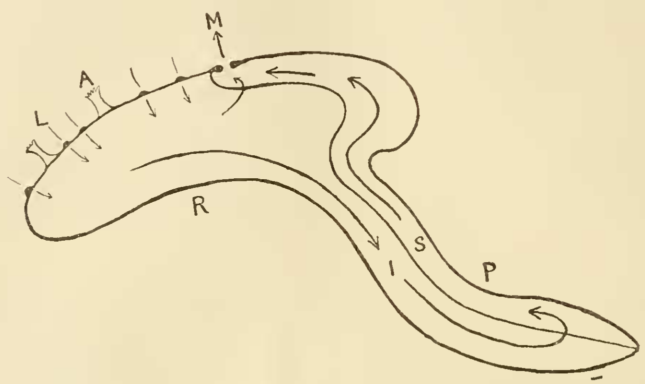

Fig. A Diagram of a median section of the rachis (*R*) of Renilla and of the peduncle (*P*) showing lateral siphonozoöids (*L*), autozoöids (*A*), inferior canal (*I*), superior canal (*S*), and pore of the median siphonozoöids (*M*). The direction of the current of water in a resting individual is shown by the arrows.

distal half where it may pass freely over into the superior canal and thence to the axial pore and out. Of these two outward courses that through the peduncle is much the freer and probably the more usual one to be followed.

An indication of the relative freedom of these two courses, as well as a check on the correctness of the hypothesis just advanced, may be gathered from the following experiments. If the peduncle of a fully distended Renilla be tied off tightly in the region where it emerges from the rachis, the animal will contract its musculature vigorously, but its volume will remain almost unaltered. Even the forceful opening of its axial pore, which during such an operation remains closed, will not allow the animal to discharge. This, however, can be accomplished immediately if the inferior canal in the rachis proximal to the ligature is cut open, or if the ligature is taken off, in which case, the axial pore immediately opens and the discharge takes place through this aperture. If the inflated animal as originally ligated be allowed to remain so, one of two things may happen. Usually it will very slowly contract till its volume has become extremely small or it will remain inflated and tense for a long time. Clearly, these two conditions indicate different degrees of freedom of connection between the superior and inferior canals in the rachides of different individuals. In some, and these must be few in number, there seems to be no connection between these two canals in the region of the rachis; in others these connections unquestionably exist, though at best they are far from being as free as those invariably present in the peduncle. One may therefore conclude that in a resting Renilla water passes from the lateral siphonozoöids to the interior system, whence it proceeds in small amounts slowly through the pores in the region of the rachis to the superior canal and in large amounts through the pores of the peduncular septum to the same canal and thus out from time to time through the pore of the axial siphonozoöid. Under such normal conditions, it is probable that none of the other apertures of Renilla, the mouths of the autozoöids and the terminal peduncular pore, are concerned in any significant way with the water current.

When such a resting animal is stimulated so that its general musculature contracts and its fluid contents are put under unusual pressure, water may then escape from it not only through the pore of the axial siphonozoöid, but through those of the lateral system and through the mouths of the autozoöids or even through the terminal pore of the peduncle.

The scheme of water circulation that has been worked out for Renilla may perfectly well apply to other sea-pens. In many of these instances, however, as Musgrave ('09) has shown, their organization is much more complicated than is that of Renilla.

Renilla, moreover, as Lightbown ('18, p. 5) declares, is probably a specialized form, hence the detailed application of such a scheme to other forms should await experimental study.

SUMMARY

1. Renilla amethystina contracts and buries itself in the sand of its natural habitat as the tide recedes, and expands above the sand when the tide returns. In contraction its volume may be diminished 88 per cent by the discharge of sea-water.

2. Sea-water enters Renilla through the lateral siphonozooids and possibly in very small amounts through the autozoöids, which certainly serve for the entrance of food. It does not enter through the axial siphonozoöid or the terminal pore of the peduncle.

3. Sea-water leaves the body of Renilla through the axial siphonozoöid which normally discharges from time to time. Under high pressure water may also escape through the lateral siphonozoöids, the autozoöids or even the terminal pore of the peduncle.

4. Within the body of Renilla the sea-water that enters by the lateral siphonozoöids collects in the inferior canal of the rachis and passes thence either by very fine openings of the peduncular septum from the inferior canal to the superior one and thus less directly but more freely out at the axial siphonozoöid, or,

5. The sea-water is drawn into the colony by the action, probably ciliary, of the lateral siphonozoöids and is expelled by general muscular contraction.

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PLATE 1

EXPLANATION OF FIGURES

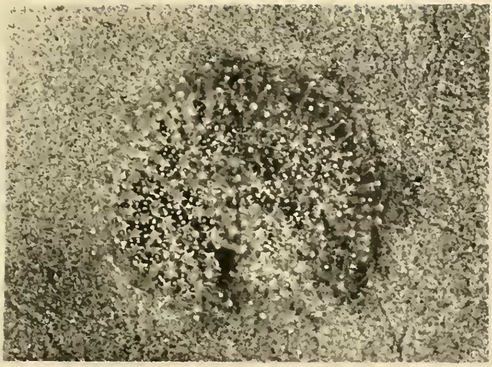
All figures represent Renilla amethystina Verrill.

1 An expanded colony of Renilla photographed in its natural position on the sand and under sea-water; seen from above.

2 A contracted colony photographed in its natural position on the sand after the withdrawal of the tide; seen from above.

3 A fully inflated colony, viewed from the inferior face.

4 A fully contracted colony seen from the side.



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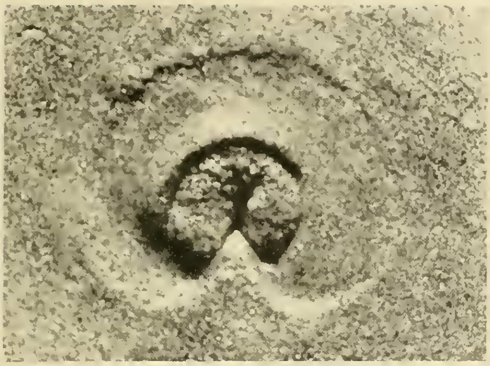

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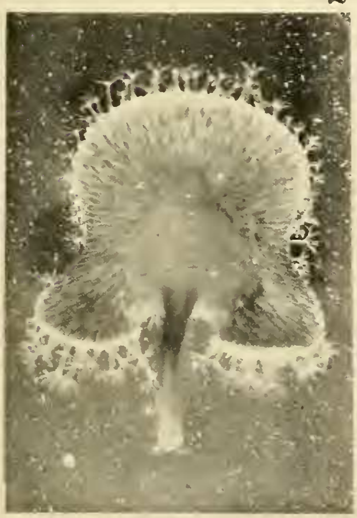

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